

Genetic experiments with a *calverleyi*-like mutation isolated from *Papilio bairdi oregonius* (Papilionidae)

David V. McCorkle

Biology Department
Western Oregon State College, Monmouth, Oregon 97361

and

Paul C. Hammond

2435 E. Applegate, Philomath, Oregon 97370

Abstract. A major aberration in the wing pattern of the black *Papilio polyxenes asterius* was discovered and named *calverleyi* in 1864. Recently a similar mutation was isolated in the yellow *P. bairdi oregonius*. Genetic experiments suggest that this trait is inherited as a simple Mendelian recessive, although possible deleterious effects from this trait may increase mortality rates among the homozygotes in certain family lines. In order to compare the *oregonius* mutation with the original *calverleyi* phenotype, it was necessary to combine the *oregonius* gene with the black wing pattern. This was accomplished by hybridizing the *oregonius* stock carrying the gene with black forms of *P. bairdi*, *P. polyxenes asterius*, and *P. joanae*, and successfully producing a phenotype nearly identical to the original *calverleyi* aberration.

Introduction

The *Papilio machaon* complex is represented in North America by many differentiated populations that have been traditionally regarded as distinct taxonomic species (Howe, 1975; Tyler, 1975). However, *P. indra* Reakirt is the only member of this group that is particularly distinct in morphology, including larval color pattern, pupal morphology, and adult male genitalic structure. In addition, artificial hybrids produced by crossing *P. indra* with other members of the *machaon* complex are apparently not viable, and did not survive beyond the first larval instar in one experiment (Emmel & Emmel, 1964).

All other taxa within the *machaon* complex are partially or completely inter-fertile (Clarke & Sheppard, 1955), and can be hybridized and back-crossed for various genetic experiments of the type reported in the present paper. Mating crosses are accomplished using the hand-pairing technique described by Clarke (1952).

Nevertheless, three or four groups of populations may be recognized as distinct biological species based upon reproductive isolation and

ecological segregation in zones of sympatry. These are outlined as follows.

1. *Papilio machaon* Linnaeus. This Eurasian species also includes two subspecies distributed in the arctic and alpine regions of Alaska and Canada. However, Sperling (1987) has recently documented extensive hybrid swarms between one of the subspecies and several members of the *P. polyxenes* group in central Canada. This suggests that these groups may be regarded as conspecific, despite the divergence in wing color pattern and allozyme patterns observed by Sperling (1987). Larval foodplants are *Artemisia arctica* Less and Umbelliferae (Tyler, 1975). *P. machaon* populations are monomorphic for the yellow color form of the adult except in hybrid suture zones.

2. *Papilio polyxenes* Fabricius. This group consists of at least seven well-differentiated subspecies or semispecies that are allopatric throughout much of North America, extending from Newfoundland to British Columbia and southward to Cuba and the Andes of South America. Larval foodplants are Umbelliferae and Rutaceae. The group is polymorphic with both yellow and black color forms in the adult, but the subspecies *asterius* Stoll used in the present experiments is monomorphic for the black form.

3. *Papilio bairdi* Edwards. This group includes four or five subspecies that are widely distributed in the arid regions of western North America, and are sympatric with members of the *polyxenes* group throughout their distribution. *Artemisia dracunculus* L. is apparently the only larval foodplant. As with the previous group, *P. bairdi* populations are polymorphic in adult color. Of those used in the present experiments, the typical subspecies consists primarily of the black form, while the subspecies *oregonius* Edwards is monomorphic for the yellow form.

4. *Papilio joanae* Heitzman. This is a local endemic restricted to central Missouri. It is weakly differentiated from *P. polyxenes asterius*, but does exhibit both reproductive isolation and ecological segregation from sympatric populations of this latter species (Heitzman, 1973). Larval foodplants of *P. joanae* are restricted to certain Umbelliferae. The species is monomorphic for the black form.

A very colorful aberration in the wing pattern of *P. polyxenes asterius* was named *calverleyi* by Grote (1864), and was illustrated in a color plate. In this variant, the black submarginal borders normally found in swallowtails of the *machaon* complex are completely absent on both the fore and hindwings, so that the yellow median area of the wings extends to and fuses with the yellow submarginal spots.

The original specimen was a male captured August, 1863 on Long Island, Queens Co., New York. A female of similar aberration type was subsequently captured in April, 1869 near Enterprise, Florida (Mead, 1869). Both specimens were illustrated in color by Edwards (1884), and the female was also illustrated by Holland (1899).



Fig. 1. Top row (left) normal *oregonius* male, (middle) "cal" *oregonius* male, (right) "cal" *oregonius* female with extensive orange. Middle row (left) normal *oregonius* female, (middle) "cal" *oregonius* female, (right) normal H3 *joanae* hybrid female (Jo-Or-Bd-TC 86-1). Bottom row (left) normal H3 *asterius* hybrid male (As-Or-Bd-TC 86-1), (middle) "cal" H3 *asterius* hybrid male (As-Or-Bd-TC 86-2), (right) "cal" H3 *joanae* hybrid female (Jo-Or-Bd-TC 86-1) with extensive orange.

On July 13, 1984, D. V. McCorkle captured a near-normal female of *P. bairdi oregonius* along the Columbia River at Celilo in Wasco Co., Oregon. The specimen displayed an unusually large amount of orange in the median area of the ventral hindwing. From the progeny of this female, a brother-sister mating (Or 84-1 F-2) was performed in an attempt to intensify the orange coloration through inbreeding. Of approximately 40 progeny produced from the sibling mating, 33 specimens were of a normal phenotype and 7 specimens were of a *calverleyi*-like phenotype (abbreviated "cal") in which the black wing borders were completely absent (fig. 1). As a consequence, the normal black and blue coloration of the wing borders is replaced by yellow and orange pigmentation. The numbers of the "cal" and normal phenotypes are not significantly different from the 3:1 ratio that we would expect by simple recessive inheritance for this mutation ($\chi^2 p < .28$). Because both of the sibling parents must have been heterozygote carriers of the trait to produce this ratio, the mutation must also have been carried by one of

Table 1. Experimental crosses used in the production of "cal" phenotypes.

Generation	Mating No.	Parentage male × female	Phenotypes	
			normal	"cal"
F2	Or 84-1 F-2	Celilo oregonius (sibling cross)	33	7
H1	Or-Bd 85-6	bairdi × Celilo oregonius (Or 84-1 F-2)	59	0
H2	As-Or-Bd 86-3	Or-Bd 85-6 × asterius	20	0
H2	Jo-Or-Bd 86-1	Or-Bd 85-6 × joanae	10	0
H3	As-Or-Bd-TC 86-1	As-Or-Bd 86-3 × Celilo oregonius (Or 86-1)	46	3
H3	As-Or-Bd-TC 86-2	Celilo oregonius (Or 86-3 F-2) × As-Or-Bd 86-3	4	1
H3	Jo-Or-Bd-TC 86-1	Jo-Or-Bd 86-1 × Celilo oregonius (Or 86-1)	38	7

the original wild parents at Celilo, either the male or the female. Although several other wild butterflies from Celilo were tested for this trait by inbreeding, the "cal" phenotype did not appear in other family lines.

Since the "cal" specimens displayed the same extensive orange coloration on the hindwings as their normal parents and wild grandparent, it was thought that this trait might be linked with the "cal" mutation, and would thus serve to identify heterozygote carriers of "cal". Unfortunately, subsequent crosses decoupled the orange coloration from the "cal" phenotype, proving that these traits are independently inherited (fig. 1).

Of course, the basic color background for the above "cal" mutation is of the yellow *oregonius* form, rather than the black *asterius* form of the original *calverleyi* specimens. For those readers unfamiliar with the genetics of the *Papilio machaon* complex, the black form is a simple Mendelian dominant over the yellow form (Clarke & Sheppard, 1955). Thus, we decided to test the hypothesis that the *oregonius* "cal" mutation is similar to or identical with the original *calverleyi* aberration in *asterius*. This was accomplished by combining the *oregonius* "cal" mutation through hybridization with the black form, and producing a black *calverleyi*-like phenotype very similar to that of the original specimens obtained by Grote and Mead in the 19th century (fig.

1). It should be noted that the original Mead female is of the early spring form with a well developed yellow median band and discal bar. By contrast, our specimens are of the summer form in which these yellow markings are mostly absent in black females.

As shown in Table 1, we crossed our Celilo *oregonius* stock carrying the "cal" trait with a black *P. bairdi bairdi* Edwards stock that was originally obtained from near Flagstaff, Arizona. In the first hybrid generation (H1), all progeny were of a normal phenotype. Because we were also conducting an unrelated experiment with these butterflies, we crossed the H1 *bairdi* X *oregonius* hybrids with *P. polyxenes asterius* from Warsaw in Benton Co., Missouri. Again, the H2 progeny were all of a normal black phenotype. Next, the H2 (*bairdi* X *oregonius*) X *asterius* hybrids were back-crossed to the original Celilo *oregonius* stock (progeny of Or 84-1 F-2). Although some of our H3 broods produced only normal phenotypes, two crosses did yield the "cal" mutation combined with the black phenotype, and these closely resemble the original *calverleyi* specimens. We also replicated this experiment by substituting *P. joanae* from Warsaw, Missouri for the *asterius* parent in the H2 hybrid cross, and again obtained black *calverleyi*-like specimens in the H3 back-cross to Celilo *oregonius* (fig. 1).

The "cal" mutation may represent some type of deletion in the genetic information needed to produce the black wing borders in the *Papilio machaon* complex. This could result from a simple point-mutation at a control locus. However, it could also be the result of a major deletion of a chromosomal arm, perhaps even an entire chromosome. We have not yet attempted any karyotype studies to check this possibility. However, "cal" homozygotes appear to exhibit various pleiotropic and/or epistatic effects from this mutation in addition to the black wing borders. Most "cal" individuals show reduced vigor and poor fertility, while their normal siblings show normal vigor and fertility. As yet, we have only obtained two larvae from a "cal" homozygote (neither survived), and all of our breeding experiments have been conducted with heterozygote carriers of "cal". This has not been easy, because the carriers do not differ in phenotype from non-carriers. Moreover, in many family lines which produce "cal" phenotypes, there is often a sharp deficit in the number of "cal" homozygotes. For example, in one of our H3 hybrid back-crosses to Celilo *oregonius* (As-Or-Bd-Tc 86-1), 49 progeny were obtained, but only 3 were of the "cal" phenotype. This is a very significant deviation from the 3:1 ratio that we expected to obtain ($\chi^2 < .003$), and suggests that the homozygotes of "cal" may suffer exceptional mortality during development. Such deleterious effects may be expected if the mutation is the result of a chromosomal deletion.

In conclusion, the information that we have obtained in studying the "cal" mutation demonstrates the value of conducting inbreeding experiments with butterflies. First, these studies provide some insight into the recessive genetic variation carried by natural butterfly populations as

recently noted by Dimock & Mattoni (1986). Second, specific genetic variants provide insight into how the butterfly genome is structured and functions. In the case of the "cal" mutation, it provides us with the knowledge that a specific part of the genome is responsible for producing the black wing borders in the *Papilio machaon* complex, and that this segment of genetic information is independent of other parts of the genome that encode for the remaining components of the wing pattern including general coloration, submarginal spots, black discal bars, and the anal spot of the hindwing.

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